# HEAVY CHAIN MUTANT LEADING TO IMPROVED IMMUNOGLOBULIN PRODUCTION

This application is the National Stage of International Application No. PCT/EP2008/005136 filed Jun. 25, 2008, which claims the benefit of EP 07012774.1 filed Jun. 29, 2007, which is hereby incorporated by reference in its entirety.

The current application describes methods and nucleic acids useful in the production of immunoglobulins in mammalian cells.

## BACKGROUND OF THE INVENTION

Expression systems for the production of recombinant polypeptides are well-known and reported in the state of the art literature. For the production of polypeptides used in pharmaceutical applications mammalian cells such as CHO cells, BHK cells, NS0 cells, Sp2/0 cells, COS cells, HEK cells, PER.C6® cells, and the like are employed. The nucleic acid encoding the polypeptide is introduced into the cell e.g. in a plasmid, such as, for example, an expression plasmid. The essential elements of an expression plasmid are a prokaryotic plasmid propagation unit, e.g. for *Escherichia coli* comprising an origin of replication and a selection marker, a eukaryotic selection marker, and one or more expression cassettes for the expression of the nucleic acid(s) of interest each of them comprising a promoter, a structural gene, and a transcription terminator including a polyadenylation signal. For transient expression in mammalian cells a mammalian origin of replication, such as the SV40 On or OriP, may be included. As a promoter a constitutive or inducible promoter can be selected. For optimized transcription a Kozak sequence may be included in the 5' untranslated region. For mRNA processing a polyadenylation signal may be included as well.

Proteins and especially immunoglobulins play an important role in today's medical portfolio. For human application every pharmaceutical substance has to meet distinct criteria. To ensure the safety of biopharmaceutical agents to humans substances, which would cause severe harm, have to be removed especially.

The splicing of mRNA is regulated by the occurrence of a splice donor site in combination with a splice acceptor site, which are located at the 5' end and 3' end of an intron, respectively. According to Watson et al. (Watson et al. (Eds), Recombinant DNA: A Short course, Scientific American Books, distributed by W.H. Freeman and Company, New York, N.Y., USA (1983)) are the consensus sequence of the 5' splice donor site ag|gtragt (exon|intron) and of the 3'splice acceptor site $(y)_nNcag|g$ (intron|exon) (r=purine base; y=pyrimidine base; n=integer; N=any natural base).

In 1980 first articles dealing with the origin of secreted and membrane bound forms of immunoglobulins have been published. The formation of the secreted (sIg) and the membrane bound (mIg) isoform results from alternative splicing of the heavy chain pre-mRNA. In the mIg isoform a splice donor site in the exon encoding the C-terminal domain of the secreted form (i.e. the $C_H3$ or $C_H4$ domain, respectively) and a splice acceptor site located at a distance downstream thereof are used to link the constant region with the downstream exons encoding the transmembrane domain.

A method to prepare synthetic nucleic acid molecules having reduced inappropriate or unintended transcriptional characteristics when expressed in a particular host cell is reported in WO 2002/016944. In WO 2006/042158 are reported nucleic acid molecules modified to enhance recombinant protein expression and/or reduce or eliminate mis-spliced and/or intron read through by products.



Therefore there exists a need for a recombinant production method for immunoglobulins with reduced by products.

## SUMMARY OF THE INVENTION

The current invention comprises in a first aspect a nucleic acid encoding the amino acid sequence of the C-terminal part of the $C_H3$-domain of an immunoglobulin of the class IgA or IgG, or the C-terminal part of the $C_H4$-domain of an immunoglobulin of the class IgE or IgM, wherein the glycine-lysine-dipeptide comprised in the primary amino acid sequence of the C-terminal part of the $C_H3$- or $C_H4$-domain is encoded by the nucleic acid ggaaaa, or the nucleic acid ggcaaa, or the nucleic acid gggaaa, or the nucleic acid ggaaag, or the nucleic acid ggcaag, or the nucleic acid gggaag.

In one embodiment the nucleic acid according to the invention encodes an amino acid sequence selected from the amino acid sequences SEQ ID NO: 1, 3, 4, 5, 6, 7, or 8. In another embodiment is the nucleic acid encoding the glycine-lysine-dipeptide preceded by the nucleotide g or a. In another embodiment is the nucleic acid encoding the glycine-lysine-dipeptide the nucleic acid ggaaaa, or the nucleic acid ggcaaa, or the nucleic acid gggaaa.

The second aspect of the current invention is a plasmid comprising the nucleic acid according to the invention, and the third aspect of the invention is a cell comprising the nucleic acid according to the invention.

A further aspect of the invention is a nucleic acid with the nucleotide sequence of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 30, or 31.

The fifth aspect of the invention is a method for the production of an immunoglobulin in a mammalian cell comprising the following steps:

a) transfecting a mammalian cell with a nucleic acid encoding an immunoglobulin heavy chain comprising a nucleic acid of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 30, or 31, which encodes the C-terminal part of the immunoglobulin heavy chain,
b) cultivating the transfected mammalian cell under conditions suitable for the expression of the immunoglobulin,
c) recovering the immunoglobulin from the culture or the cell.

In one embodiment is the mammalian cell a CHO cell, a BHK cell, a NS0 cell, a Sp2/0 cell, a COS cell, a HEK cell, or a PER.C6® cell. Preferably the mammalian cell is a CHO cell, or a BHK cell, or a PER.C6® cell. In another embodiment is the mammalian cell transfected with two nucleic acids, wherein the first nucleic acid comprises an expression cassette encoding an immunoglobulin light chain, and wherein the second nucleic acid comprises an expression cassette encoding an immunoglobulin heavy chain comprising a nucleic acid of SEQ ID NO: 17, 18, 19, 20, 21, 22, 23, 30, or 31 encoding the C-terminal part of the immunoglobulin heavy chain.

The final aspect of the invention is a method for improving the expression of an immunoglobulin in a mammalian cell, wherein the nucleic acid encoding the immunoglobulin heavy chain comprises the nucleic acid ggaaaa, or the nucleic acid ggcaaa, or the nucleic acid gggaaa, or the nucleic acid ggaaag, or the nucleic acid ggcaag, or the nucleic acid gggaag encoding the glycine-lysine-dipeptide contained in the $C_H3$- or $C_H4$-domain.

## DETAILED DESCRIPTION OF THE INVENTION

The current invention comprises a nucleic acid encoding the amino acid sequence of the C-terminal part of the $C_H3$-